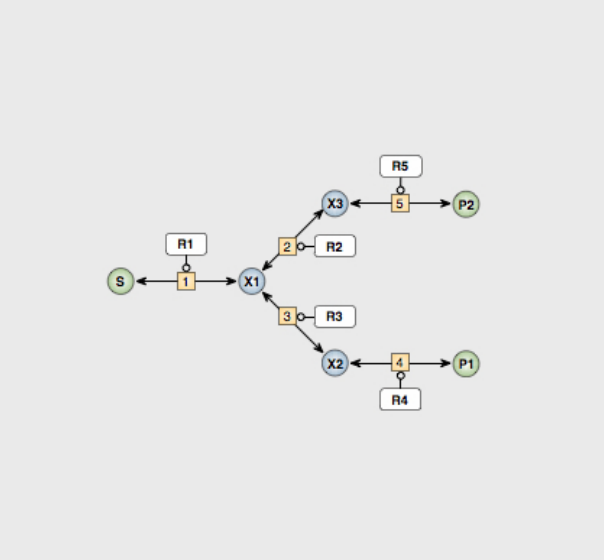
Systems Biology Tutorial 4:
Structural analysis of reaction networks — MEMO

1. Consider the branched pathway:



(a) Construct the stoichiometric matrix **N** by hand.

$$\mathbf{N} = \begin{bmatrix} 1 & -1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 1 & 0 & 0 & -1 \end{bmatrix} \quad \text{where} \quad \frac{d}{dt}\begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix} = \mathbf{N} \cdot \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix}$$

(b) Are there any dependent metabolites?
No.

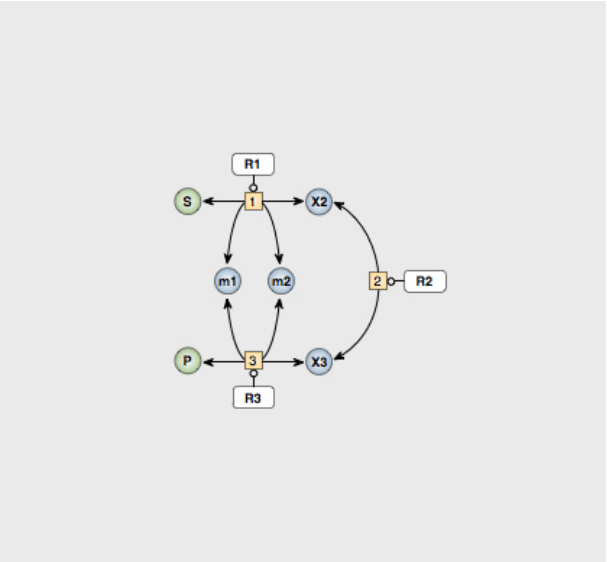
(c) Derive the steady-state flux relations by hand from $\mathbf{Nv} = \mathbf{0}$. How many independent fluxes are there?
2 independent fluxes. If you choose J_4 and J_5, the relations are:

$$J_1 = J_4 + J_5$$
$$J_2 = J_5$$
$$J_3 = J_4$$

(d) Load the model `branch5.psc` into PySCeS. Obtain the **N**, **K** and **L** matrices with the commands `mod.showN()`, `mod.showK()` and `mod.showL()`. Show the flux relationships with `mod.showFluxRelationships()`. Show the metabolite conservation relationships with `mod.ShowConserved()`.

(e) Check your answers by running the `branch5` model on JWS Online (`http://jjj.biochem.sun.ac.za/models` or `http://jjj.bio.vu.nl/models`) and generating the **N**, **K** and **L** matrices.

2. Consider the linear pathway with a moiety:



(a) Construct the stoichiometric matrix **N** by hand.

$$\mathbf{N} = \begin{bmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 1 & 0 & -1 \\ 0 & 1 & -1 \end{bmatrix} \quad \text{where} \quad \frac{d}{dt}\begin{bmatrix} m_1 \\ x_2 \\ m_2 \\ x_3 \end{bmatrix} = \mathbf{N} \cdot \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix}$$

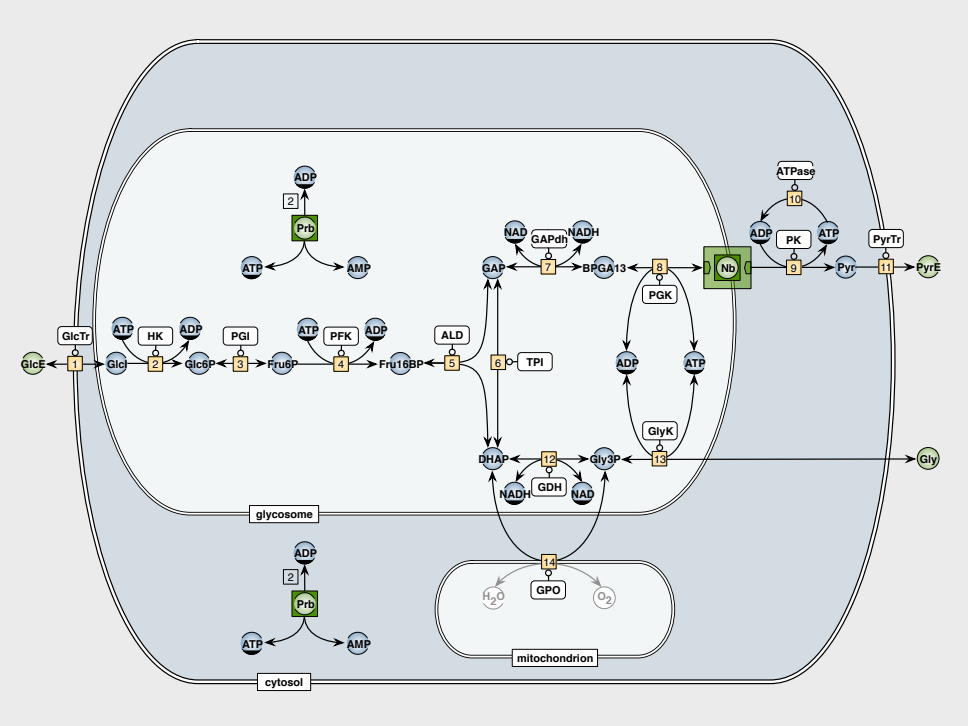
(b) Are there any dependent metabolites?
Yes. $m_1 + m_2 = C_1$ *and* $m_1 + x_2 + x_3 = C_2$, *where* C_1 *and* C_2 *are the moiety-conserved sums. Choose* m_1 *and* x_2 *as independent metabolites, then* m_2 *and* x_3 *are dependent metabolites. (Other combinations are possible.)*

(c) Derive the steady-state flux relations by hand from $\mathbf{Nv} = \mathbf{0}$. How many independent fluxes are there?
1 independent flux. $J_1 = J_2 = J_3$

(d) Using the PSC file from Question 1 as template, create a new PSC file for this pathway and save it as `lin3moi.psc`. Load this model into PySCeS and obtain the **N**, **K** and **L** matrices as in Question (1d).

(e) Check your answers by running the `lin3moi` model on JWS Online and generating the **N**, **K** and **L** matrices.

3. Consider the following model for glycolysis in *Trypanosoma brucei*. The PSC file for this model is available for download (`Bakker.psc`), and you can run it on JWS Online (`bakker`).



(a) How many independent fluxes are there?
2 (we can choose J_7 and J_{12}, but other combinations are possible)

(b) Assuming anaerobic glycolysis ($J_{14} = 0$, i.e. no flux through v_{GPO}):

i. What is the flux relation between
- J_{12} and J_7,
  $J_{12} = J_7$
- J_{13} and J_1?
  $J_{13} = J_1$ *(because* $2 \times J_1 = J_7 + J_{12}$ *and* $J_{12} = J_7$ *and* $J_{12} = J_{13}$*, thus* $2 \times J_1 = 2 \times J_{13}$*)*

ii. What is the ratio of PyrE to Gly produced?
1:1

iii. How many moles of ATP are produced per mole of Glc in the glycosome?
Net production is 0. v_2 consumes 1, v_4 consumes 1, v_8 produces 1, v_{13} produces 1.

iv. How many moles of ATP are produced per mole of Glc in the cytosol?
Net production is 1 (v_9).

(c) Assuming aerobic glycolysis ($J_{14} \neq 0$):

i. Production of which product will increase?
PyrE, since reaction v_{14} will decrease the flux towards Gly.

ii. What is the maximal flux through J_7 related to J_1?
$J_7 = 2 \times J_1$

iii. How many moles of ATP are produced per mole of Glc in the glycosome (maximally)?
Net production is 0. v_2 consumes 1, v_4 consumes 1, v_8 produces 2.

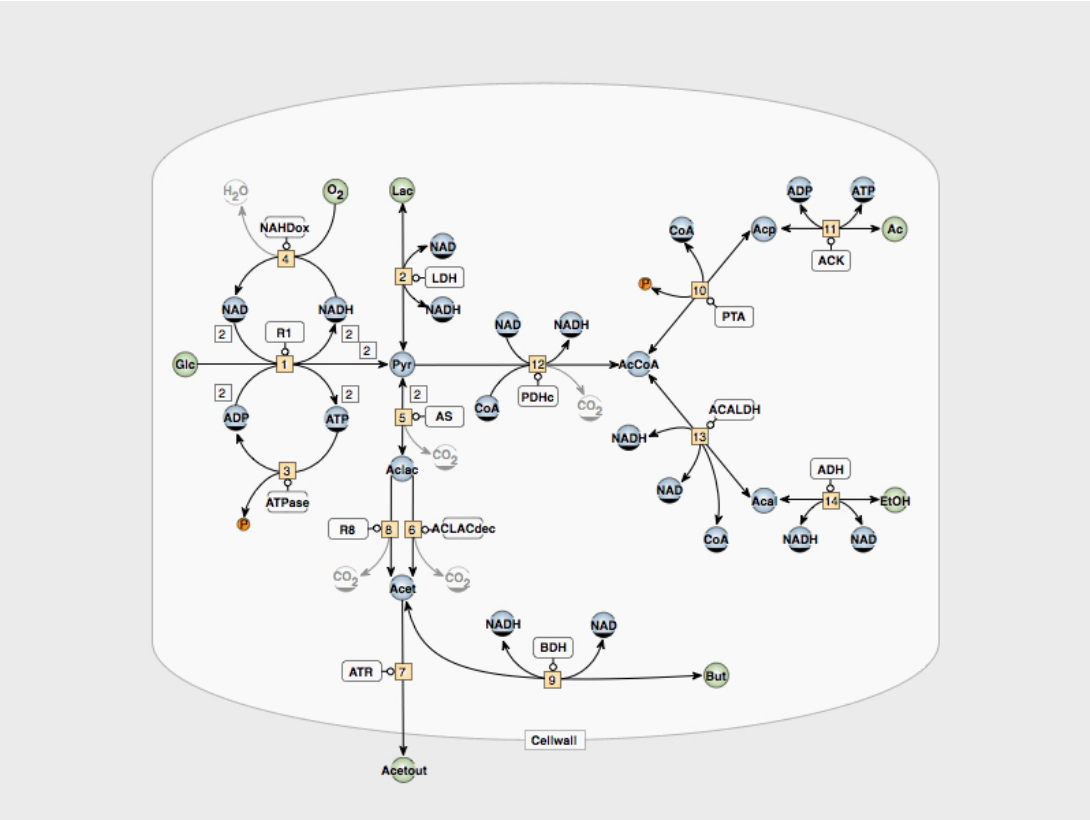
iv. How many moles of ATP are produced per mole of Glc in the cytosol (maximally)?
Net production is 2 ($J_9 = 2 \times J_1$ maximally).

(d) Test your answer by running the `Bakker.psc` model in PySCeS. Adjust the V_{max} of GPO (`Vm9` in the model) to simulate anaerobic/aerobic conditions, calculate the steady state and display the results with `mod.showState()`.

(e) You can also run the model in JWS Online. Do the results agree?

(f) Would glycolysis reach a steady state in the absence of the glycerol branch? Why?
No. Redox balance will not be maintained, i.e. the organism will run out of NAD (all the NAD/NADH moiety will be in the form of NADH).

4. Consider the following model for glycolysis in *Lactococcus lactis*. The numbers $\boxed{2}$ are stoichiometric coefficients. Unless indicated in this way, all other stoichiometries are 1. The PSC file for this model is available for download (`Hoefnagel1.psc`), and you can run it on JWS Online (`hoefnagel1`).



(a) Consider anaerobic glycolysis ($v_{NADHox} = 0$):

i. Would the production of lactate be possible for anaerobic glycolysis? If so, what would be the flux relation bewteen J_2 and J_1 if all available pyruvate is converted tot lactate? How many moles of lactate will be produced per mole of glucose?
Yes. $J_2 = 2 \times J_1$. 2 mol of lactate per mol glucose.

ii. How much EtOH will be formed per mole of glucose if all pyruvate were converted to AcCoA?
Anaerobic conditions, $\Rightarrow$ redox balance has to be mantained in pathway. Up to AcCoA there are 4 NADH and 2 AcCoA produced per Glc. All AcCoA therefore has to be converted to EtOH in order to re-oxidise the NADH to NAD (2 NADH per AcCoA). Therefore only EtOH will be formed (2 EtOH per Glc).

(b) Consider aerobic glycolysis ($v_{NADHox} \neq 0$):

i. Which branch would maximize ATP production?
Redox balance is no longer a problem. All Glc converted to Ac would therefore result in maximum ATP production (4 ATP per Glc).

ii. How many moles of product will be formed per mole of glucose if this branch was carrying all the flux?
2 mol Ac per mol Glc.

(c) Is the production of butanol redox neutral?
No. v_1 converts 2 NAD to 2 NADH whereas v_9 converts only 1 NADH to 1 NAD.

(d) What would be the ratio of Ac to EtOH in the case of anaerobic glycolysis in *E. coli* if v_{12} does not consume NAD under these conditions?
Anaerobic conditions, $\Rightarrow$ redox balance has to be mantained in pathway. Up to AcCoA there are now 2 NADH and 2 AcCoA produced per Glc. The two AcCoA can therefore split equally between EtOH and Ac. The ratio EtOH:Ac is therefore 1:1.